Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigation of **Calcium Ascorbate** in the developing chicken embryo 12/29/77

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: December 29, 1977

THRU : HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicity Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigation of Calcium Ascorbate in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Calcium Ascorbate

Protocol:

Calcium Ascorbate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of colums 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Calcium ascorbate was moderately toxic to the chicken embryo. Treatment via the air cell at 0 hours between 10 and 200 mg/kg resulted in a regression line whose slope was not significantly different from zero ($p=0.05$). At 96 hours, the calculated LD_{50} was 43.68 mg/kg (2.18 mg/egg). Yolk treatment at 0 hours gave a calculated LD_{50} of 102.19 mg/kg (5-11 mg/egg, while at 96 hours the slope of the line was not significantly different from zero ($p=0.05$).

Scattered abnormalities were observed under all conditions of test, but except for one instance (200 mg/kg, air cell at 0 hours) serious abnormalities were not significantly higher than or different from those observed in the background. Calcium ascorbate displayed no teratogenicity under the test conditions employed.

1. Calcium Ascorbate, Pfizer Chem. Div. of New York, Lot # GC3240-C3024

2. McLaughlin, J., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M.J., Marliac, J.P., and McLaughlin, J., Jr., (1964) JOAC 47, 1002-1006

4. Finnery, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

TABLE 1

Calcium Ascorbate

Air Cell @ 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	120	56.66*	10.00*	5.83*
5.00	100.00	120	38.33*	5.83	3.33
2.50	50.00	119	35.29	2.52	2.52
1.250	25.00	120	36.66*	5.83	1.66
0.50	10.00	120	35.83	5.83	1.66
Water		120	24.16	0.83	0.00
Controls		306	7.51	1.96	1.96

* Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero p=0.05

TABLE 2

Calcium Ascorbate

Air Cell @ 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	115	80.00*	4.34	0.86
2.50	50.00	115	56.52*	4.34	1.73
1.250	25.00	115	41.73*	6.08	3.47
0.6250	12.50	114	28.94*	2.63	3.50
0.250	5.00	115	27.82*	6.08	1.73
Water		120	15.00	4.16	3.33
Controls		306	7.51	1.96	1.96

* Significantly different from solvent $p \leq 0.05$

**LD_{50} 43.677 mg/kg (2.184 mg/egg)

TABLE 3

Calcium Ascorbate

Yolk @ 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	119	67.22*	2.52	0.00
5.00	100.00	120	65.83*	0.83	0.83
2.50	50.00	120	60.00*	2.50	0.83
1.250	25.00	120	54.16*	0.83	0.83
0.50	10.00	120	50.00*	0.00	0.00
Water		120	28.33	2.50	1.66
Controls		306	7.51	1.96	1.96

* Significantly different from solvent $p \leq 0.05$

** LD_{50} 102.193 mg/kg (5.11 mg/egg)

TABLE 4

Calcium Ascorbate

Yolk @ 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	115	40.00*	4.34	2.60
2.50	50.00	145	41.37*	1.37	1.37
1.250	25.00	115	35.65*	3.47	3.47
0.6250	12.50	115	40.00*	6.08	6.08
0.250	5.00	85	31.76*	0.00	0.00
Water		119	15.12	0.84	0.84
Controls		306	7.51	1.96	1.96

* Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero p=0.05